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TECHNICAL MANUSCRIPT 200

A DEVICE FOR CREATING DRY AEROSOLS FOR EXPERIMENTAL INFECTIVITY STUDIES

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CORRECTION: Dr. Beebe's name should be corrected to read

James M. Beebe

U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

TECHNICAL MANUSCRIPT 200

A DEVICE FOR CREATING DRY AEROSOLS FOR EXPERIMENTAL INFECTIVITY STUDIES

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ABSTRACT

Equipment is needed that will permit laboratory studies with experimental animals using aerosols of dusts, spores, microorganisms, or toxins. This report describes a machine capable of producing dynamic clouds and suitable for attachment to animal exposure boxes.

A dry preparation of *Serratia marcescens* with 68.0% of the particles less than 5 μ in diameter was used for calibration. Test material compacted in a small cartridge was forced by plunger action onto the serrated edge of a metering wheel, which then presented the powder to a dry air jet, creating a cloud. Samples were collected by impinging the particles in a liquid. Viability was determined by spreading dilutions of that liquid on agar plates. The distribution of particle diameters was determined by measuring at least 500 particles collected on microscope slides.

Plunger speed was found to have a highly significant effect on particle concentration in the aerosol at the slow speed of the metering wheel. An approximate maximum fivefold difference in cloud concentration (from 3.9×10^6 to 21.8×10^6 viable *S. marcescens* cells) was found by testing the various plunger and metering wheel agar combinations. Approximately 97% of the particles generated in the experimental aerosols were less than 5 μ in diameter.

A DEVICE FOR CREATING DRY AEROSOLS FOR EXPERIMENTAL INFECTIVITY STUDIES

Investigations of the toxicity of soluble substances and the infectivity and virulence of microorganisms by exposure of laboratory animals to aerosols of such materials have largely been confined to "clouds" generated from liquid suspensions. However, many diseases and pathological conditions result from the inhalation of dry microorganisms, dusts, and toxic materials. It would be highly desirable to have equipment that would permit laboratory studies with experimental animals using dusts, spores, microorganisms, or toxins prepared in an aerosol of finely divided dry powder. The lack of a suitable device for continuously generating an aerosol from dried materials has hindered such investigations. This report discusses a study of a small machine that is capable of producing dynamic clouds of dry materials; and this device can be attached to animal exposure boxes. Because the concentration of the cloud could be varied by means of gears, the experimental conditions, number of particles counted, and determination of viability of bacterial cells were replicated so that the results could be analyzed statistically.

The device disseminates the aerosol from a column of packed powder that is forced by a plunger onto the serrated edge of a metering wheel. The compacted powder is removed in small increments by the metering wheel and is presented to a high-velocity air jet that generates the aerosol at a rate of 8 liters per minute. The rate of plunger travel and speed of the metering wheel are controlled by separate sets of gears. In order to observe the behavior of clouds thus produced, the disseminating device was attacked to a small animal holding box, one of several different kinds used with a Henderson apparatus in our laboratory for respiratory infection studies. Figure 1 is a photograph of the disseminator attached to the animal holding box with a Shipe aerosol sampler in position. The Shipe sampler collects the disseminated powder by impinging the particles in a liquid. Figure 2 is a close-up view of the gear trains that control the rate of plunger travel and speed of the metering wheel; the aerosol exit port and the connection for compressed air are shown in Figure 3.

A dry preparation of *Serratia marcescens* with 68.0% of the particles less than 5 μ in diameter was used to calibrate the disseminator. This powder was packed in a small (0.746cc) cartridge-type magazine by the use of weights. The magazine was held in alignment with a piston in a simple device that permitted dry powders to be compacted to various degrees by

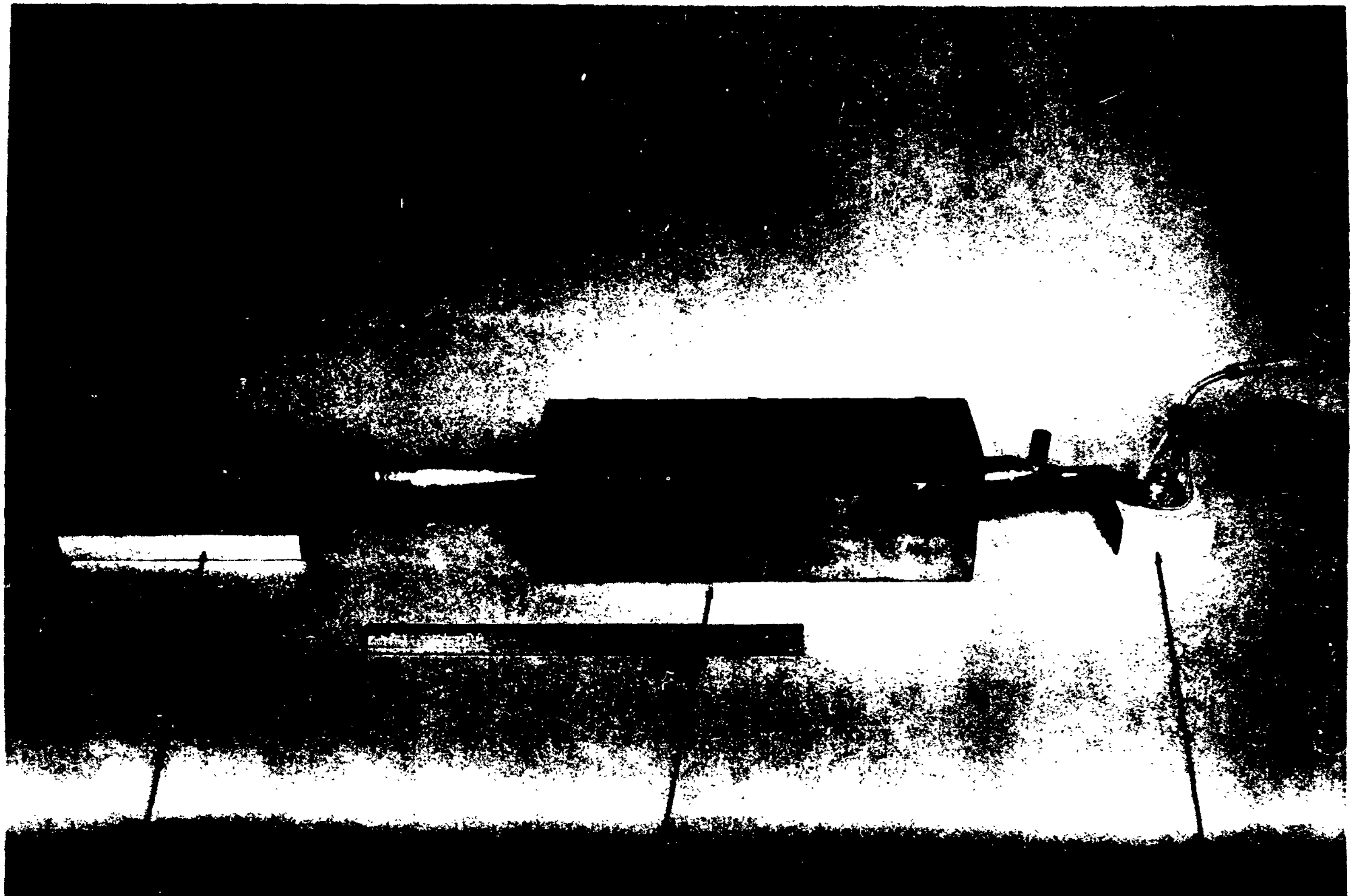

Figure 1. Dry Aerosol Disseminator Attached to Animal Exposure Box.

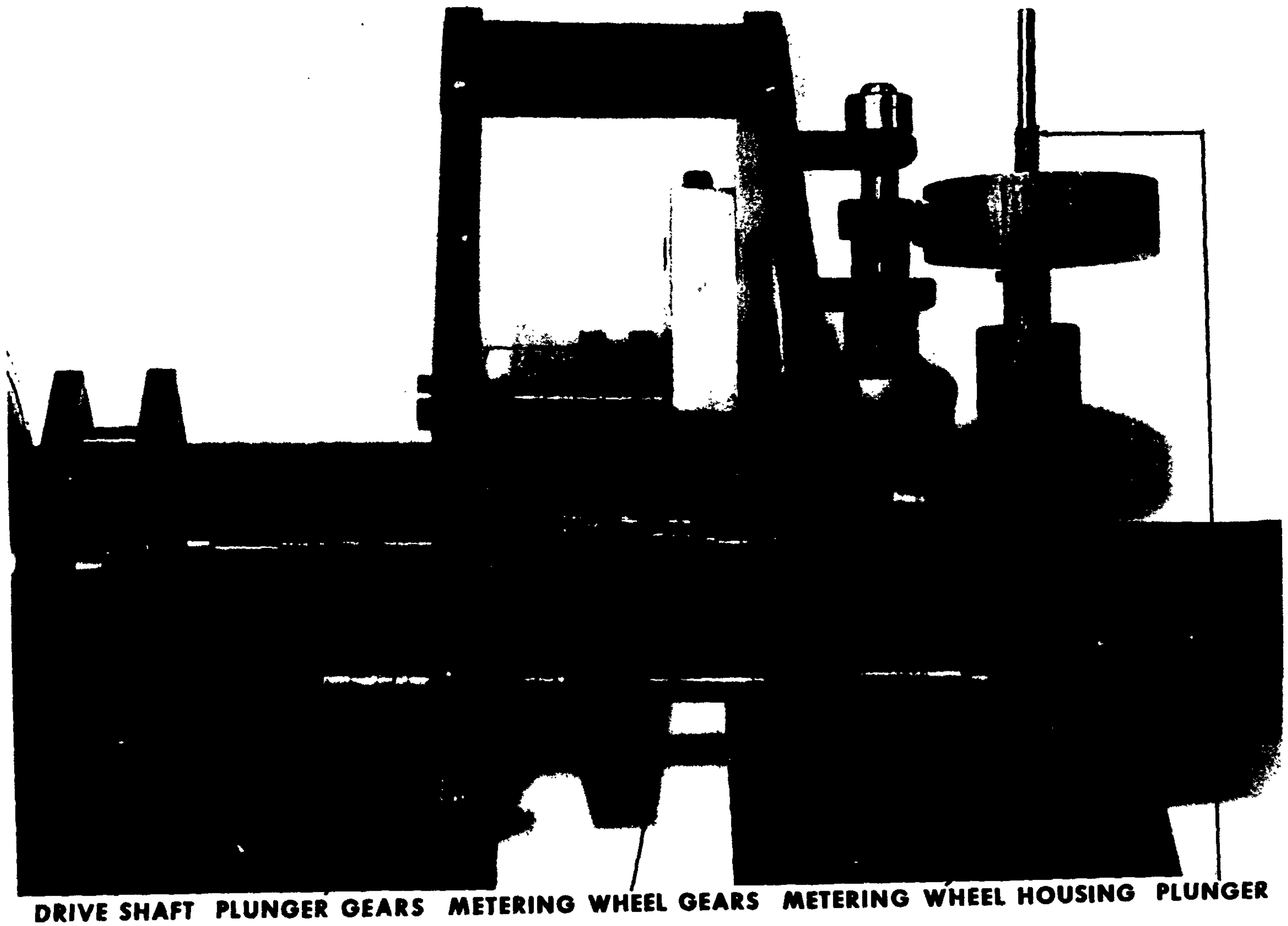

Figure 2. Close-Up View of Dry Aerosol Disseminator Gear Trains.

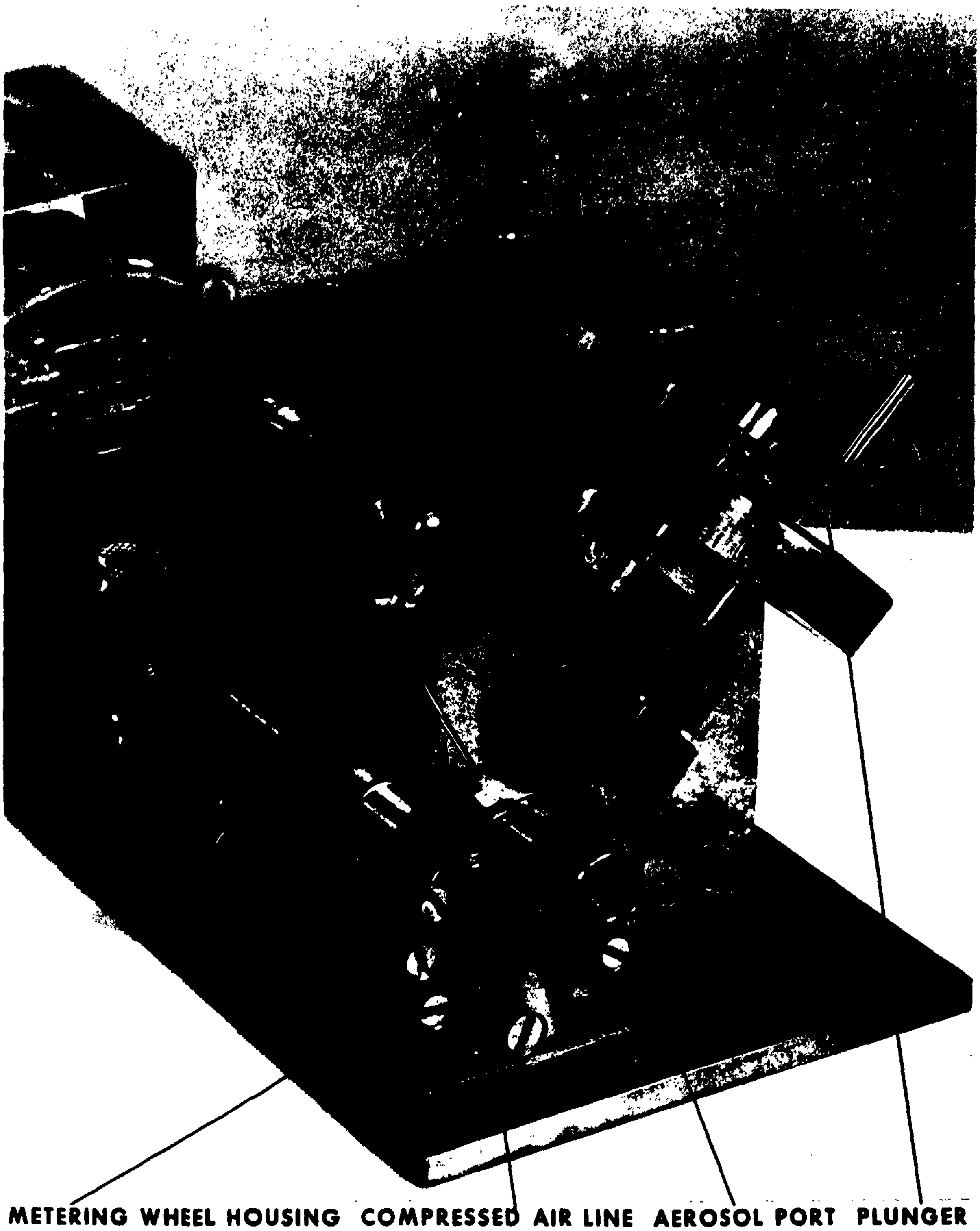

Figure 3. Dry Aerosol Disseminator Showing Connection for Compressed Gas, Plunger Mechanism, and Aerosol Exit Port.

placing weights on top of the piston, as shown in Figure 4. Weight in excess of 700 grams packed the test material too tightly, but 200- to 700-gram weights provided a uniform cohesive column. A quantity of the powdered material sufficient for the entire investigation was kept under refrigeration, and an amount sufficient for one day's testing was removed on each of nine working days. Decimal dilutions of 0.1 gram of test material in tryptose saline diluent were spread on replicate tryptose agar plates at the beginning and end of each working day for viability determinations.

The cloud was initiated by manual movement of the plunger until the cloud became visible in a Tyndall beam. The machine was then operated mechanically for a one-minute equilibration period before a one-minute cloud sample was collected. After the impingers were changed, the machine was restarted and a second one-minute equilibration period was allowed before a second one-minute sample was collected. A third sample was collected in like manner. After 30 such runs, the data showed that counts of the first impinger sample in each run were somewhat higher than those of the second and third samples. Therefore, a two-minute equilibration period was employed for runs 31 through 54. Cloud samples were collected in Shipe impingers containing 25 ml of tryptose saline solution at a flow rate of 12.5 liters per minute. Impinging fluids were diluted and spread on tryptose agar plates to determine viable cell numbers.

Between the first and second impinger samples, a cloud sample was taken to determine particle size distribution. This was accomplished by placing a clean glass slide, bearing no adhesive, on the bottom inside a one-gallon paint can, passing the aerosol through the can for 3 minutes, and then allowing the aerosol to settle 48 hours. At least 500 particles per slide, which comprised 20 to 30 fields, were measured under high dry magnification by comparison with a series of calibrated circles on a Howard disc incorporated into the eyepiece of a microscope. Alterations were made in plunger and metering wheel speeds by changing gear settings prior to each run, in order to assess a range of cloud concentrations. Between runs the machine was partly disassembled and parts in contact with test material (and the animal holding box) were cleaned in 95% ethyl alcohol. After reassembly, an operating period of one minute without aerosol generation assured the complete evaporation of residual alcohol. Air was by-passed through the animal holding box at 20 liters per minute. Immediately before each run, a one-minute sample of that air was collected to make certain that no significant contamination from the previous run was present. This contamination, or carry-over from one run to the next, when present, had a concentration approximately four to six logarithms less than the experimental clouds and was ignored in the statistical analysis.

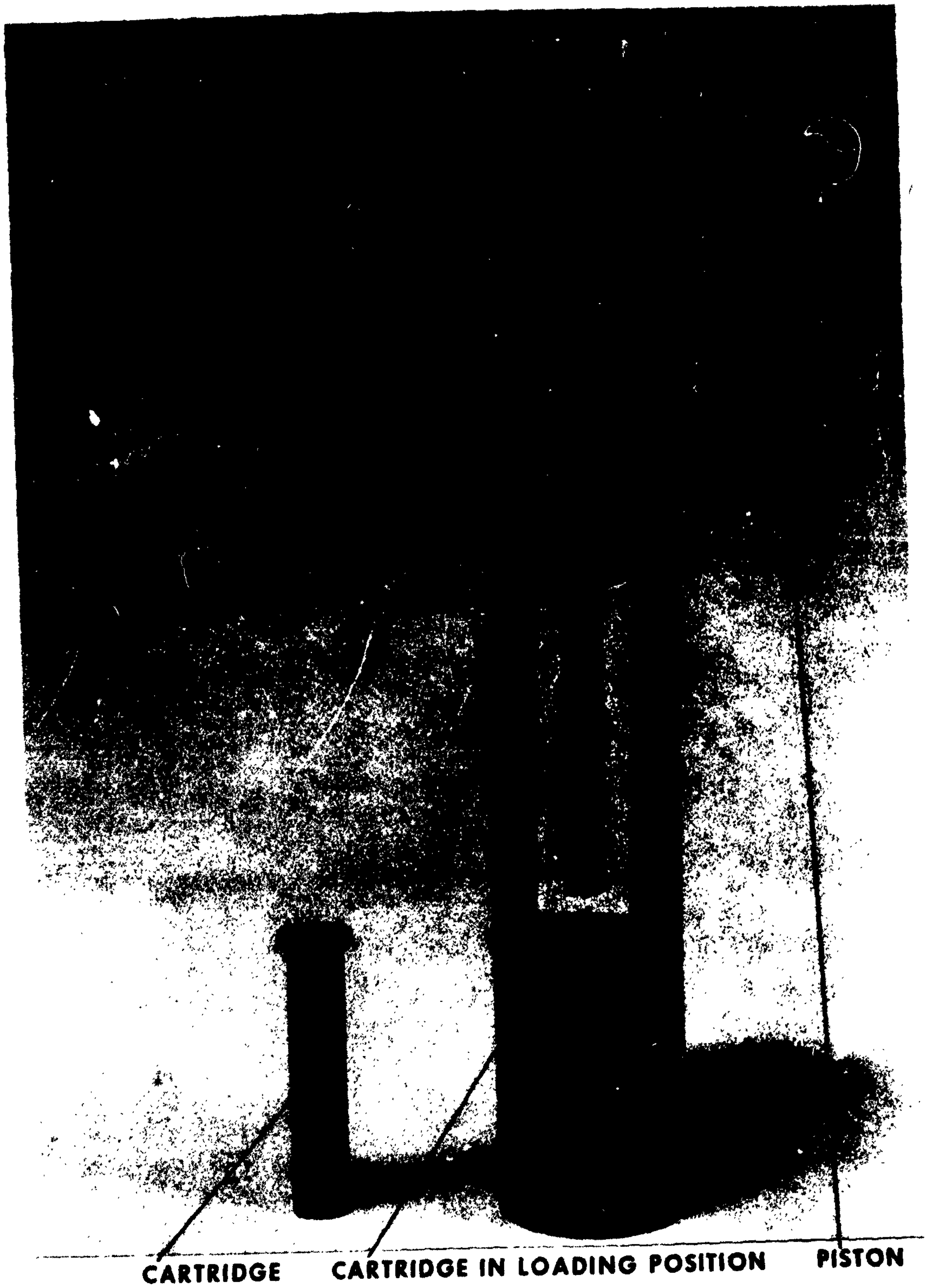

Figure 4. Device for Compacting Dry Test Materials.

One complete replication of this experiment required 18 runs; that is, 3 metering wheel speeds by 3 plunger speeds by either 200 or 700 grams used for compaction of test material. Only six runs per day could be accomplished efficiently. A split plot design was utilized, wherein wheel speed was considered as the whole plot, and the subplot was composed of the factorial arrangement of plunger speed and compaction weight. In this particular design, wheel speed is completely confounded, with day effects, which were assumed to be negligible. Wheel speeds were randomized within each replication, and combinations of plunger and compaction treatment were randomized within each day. Table 1 shows the order in which the runs were conducted.

Plunger speed was found to have a highly significant effect on cloud concentration at the slow metering wheel speed, but effects due to wheel speed and compaction weights were not detected. The interaction of plunger and wheel speeds indicated that the plunger effect was not the same with all wheel speeds. This is illustrated in Table 2, which shows that the plunger has its greatest effect at slow wheel speed, and that at the fast wheel speed the plunger had no effect. Plunger effect on cloud concentration at the intermediate wheel speed gave values between those found as the fast and slow wheel speeds. An approximate maximum fivefold difference in cloud concentration (from 3.9×10^6 to 21.8×10^6 viable Serratia marcescens cells) was found by testing these various combinations of plunger and metering wheel gear.

Plunger speed had a statistically significant linear effect on mean particle size and wheel speed had a quadratic effect. Figure 5 shows the mean relative frequency as per cent of each size classification by plunger speed.

The smallest comparison circle on the Howard ocular disc measured 1.4 μ, which prevented recording particles smaller than this figure. The detection of very large particles would have required examination of many more than 20 to 30 fields for full representation.

This machine has a potential application in many areas of investigation involving aerosols of small dry particulates. Respiratory infectivity or toxicity could be studied for many biological materials, such as bacterial or fungal spores and various toxic materials. Inorganic chemical fumes, smokes, or dusts could be assayed in the dry state, provided that they were not too corrosive or abrasive for the machine. Patent rights are pending on the dry aerosol disseminator described.

TABLE 1. SPLIT PLOT DESIGN AND ORDER IN WHICH TRIALS WERE CONDUCTED

	Run	P[a]	C[b]	Run	P	C	Run	P	C
	Day 1 Wheel 7[c]			Day 2 Wheel 1			Day 3 Wheel 4		
Rep 1	1	1	2	7	1	2	13	3	7
	2	3	2	8	1	7	14	1	2
	3	1	7	9	3	7	15	3	2
	4	6	2	10	6	2	16	6	2
	5	3	7	11	6	7	17	6	7
	6	6	7	12	3	2	18	1	7
	Day 4 Wheel 1			Day 5 Wheel 4			Day 6 Wheel 7		
Rep 2	19	6	7	25	3	2	31	1	2
	20	3	7	26	1	2	32	6	2
	21	1	7	27	6	2	33	6	7
	22	1	2	28	1	7	34	3	7
	23	6	2	29	6	7	35	3	2
	24	3	2	30	3	7	36	1	7
	Day 7 Wheel 4			Day 8 Wheel 7			Day 9 Wheel 1		
Rep 3	37	1	2	43	3	7	49	6	2
	38	6	7	44	1	2	50	1	7
	39	3	2	45	6	2	51	3	7
	40	3	7	46	3	2	52	1	2
	41	6	2	47	6	7	53	6	7
	42	1	7	48	1	7	54	3	2

a. P = Plunger Gear no.
b. C = Compaction Weight, Hundred grams.
c. Wheel = Metering Wheel Gear no.

TABLE 2. AEROSOL CONCENTRATIONS ATTAINED WITH DISSEMINATOR USING DRY, SIZED *SERRATIA MARCESCENS*

Metering Wheel	Viable cells per liter of cloud, 10^8		
	No. 1 Plunger Gear, Fast (1.54 mm/minute)	No. 3 Plunger Gear, Intermediate (0.64 mm/minute)	No. 6 Plunger Gear, Slow (0.19 mm/minute)
Fast (6.0 rpm)	9.6	5.7	7.3
Intermediate (1.8 rpm)	16.2	6.6	5.3
Slow (0.5 rpm)	21.8	5.0	3.9

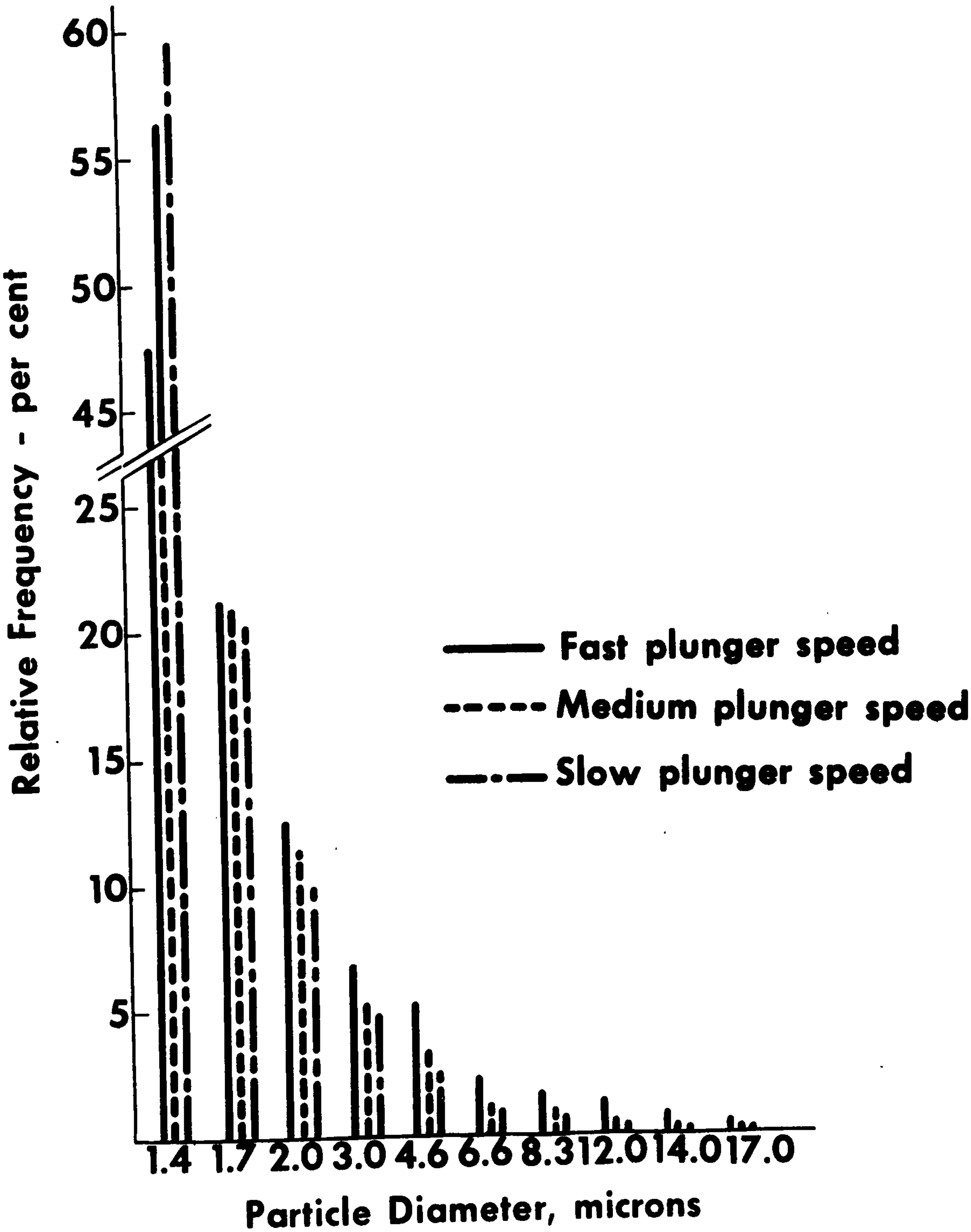

Figure 5. Mean Relative Frequency (per cent) of each Size Classification by Plunger Speed.

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